

EXHIBIT 1

BOX 15-1 Bacterial "Super-Antigens"

Staphylococcal enterotoxins are exotoxins produced by the gram-positive bacterium *Staphylococcus aureus* and consist of five serologically distinct groups of proteins: SEA, SEB, SEC, SED, and SEE. These toxins are the most common cause of food poisoning in humans. A related toxin, TSST, causes a disease called toxic shock syndrome (TSS), which is associated with tampon use. Pyrogenic exotoxins of streptococci and exotoxins produced by mycoplasmas may be structurally and functionally related to these enterotoxins.

The immune response to staphylococcal enterotoxins and related proteins has a number of features that make these bacterial products quite unique in terms of biologic and pathologic effects:

- **Staphylococcal enterotoxins are among the most potent naturally occurring T cell mitogens known.** They are capable of stimulating the proliferation of normal T lymphocytes at concentrations of 10^{-8} M or less. As many as one in five normal T cells in mouse lymphoid tissue or human peripheral blood may respond to a particular enterotoxin.
- **Enterotoxins bind to the V_β region of T cell receptors (TCRs), and each toxin stimulates T cells that express antigen receptors whose V_β regions are encoded by a single V_β gene or gene family.** In other words, the specificity of T cells for different enterotoxins is encoded in the V_β region and is not related to other components of the TCR such as the V_α , J, or D segments because enterotoxins bind directly to the β chains of TCR molecules close to but outside the antigen-binding (complementarity-determining) regions. Different enterotoxins stimulate T cells expressing V_β genes from different families (see Table). Because T cells expressing only certain TCRs recognize and respond to each enterotoxin, these proteins are called antigens and not polyclonal mitogens. However, because the frequency of enterotoxin-responsive T cells is much higher than the frequency of cells specific for conventional protein antigens, the enterotoxins have been named "super-antigens."

Super-antigens bind to class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) without a need for intracellular processing and to a region of the MHC molecule away from the peptide-binding cleft. This complex is then recognized by T cells expressing antigen receptors with a particular V_β segment. The same enterotoxin binds to class II molecules of different alleles, thus indicating that the polymorphism of the MHC does not influence

the presentation of these antigens. Each staphylococcal enterotoxin molecule possesses two binding sites for class II MHC molecules. This characteristic allows each enterotoxin molecule to cross-link MHC molecules on APCs, and the enterotoxin-MHC molecule dimer may cross-link two antigen receptors on each T cell and thus initiate T cell responses.

The high frequency of staphylococcal enterotoxin-responding T cells, particularly $CD4^+$ cells, has several functional implications. Acutely, exposure to high concentrations of enterotoxin leads to systemic reactions such as fever, disseminated intravascular coagulation, and cardiovascular shock. These abnormalities are probably mediated by cytokines, such as tumor necrosis factor (TNF), produced directly by the T cells or by macrophages that are activated by the T cells. In many respects these reactions resemble systemic reactions to endotoxin (lipopolysaccharide), as in septic shock, which are also mediated by cytokines (see Chapter 12, Box 12-1). The secretion of large amounts of inflammatory cytokines is the likely pathogenesis of TSS, which is characterized by shock, skin exfoliation, conjunctivitis, and severe gastrointestinal upset and can progress to renal and pulmonary failure and death. Prolonged administration of enterotoxins to mice results in wasting, thymic atrophy, and profound immunodeficiency, also probably secondary to chronic high levels of cytokine production (e.g., TNF).

Staphylococcal enterotoxins are useful tools for analyzing T lymphocyte development. Administration of SEB to neonatal mice leads to intrathymic deletion of all immature T cells that express the $V_\beta 3$ and $V_\beta 8$ TCR genes. This deletion mimics self antigen-induced negative selection of self-reactive T cells during thymic maturation. Super-antigens also induce Fas- or TNF receptor-mediated activation-induced death of mature $CD4^+$ and $CD8^+$ T cells (see Chapter 10, Box 10-1). This type of cell death is a model of deletion of mature T cells that are repeatedly stimulated by self antigens.

Viral gene products may also function as super-antigens. In certain inbred strains of mice, different mouse mammary tumor virus genes have become incorporated into the genome. Viral antigens produced by the cells of one strain are capable of activating T lymphocytes from other strains that express particular V_β segments in their antigen receptors. The result is a form of "mixed lymphocyte reaction" that is not caused by MHC disparity. It was discovered long before viral super-antigens were identified, and the interstrain reactions were attributed to Mls (minor lymphocyte stimulating) loci. We now know that Mls "loci" are actually different retroviral genes that are stably inherited in different inbred strains.

Section
V **V_β Expression in Responding T Cells**

| Enterotoxin | Mice | Humans |
|-------------|--------------------------|----------------------------------|
| SEB | $V_\beta 7, 8.1-8.3, 17$ | $V_\beta 3, 12, 14, 15, 17, 20$ |
| SEC 2 | $V_\beta 8.2, 10$ | $V_\beta 12, 13, 14, 15, 17, 20$ |
| SEE | $V_\beta 11, 15, 17$ | $V_\beta 5.1, 6.1-6.3, 8, 18$ |
| TSST-1 | $V_\beta 15, 16$ | $V_\beta 2$ |

Abbreviations: SE, staphylococcal enterotoxin; TSST, toxic shock syndrome toxin.